

# The virgin population of *Neisseria gonorrhoeae* in Stockholm has decreased and antimicrobial resistance is increasing

M Bäckman, K Jacobson, S Ringertz

## Abstract

**Aims**—To investigate the evolution of chromosomal and plasmid mediated resistance for ampicillin and tetracycline of *N gonorrhoeae* strains in Stockholm during 1982–1993.

**Methods**—A total of 404 gonococcal strains isolated in 1982, 1987, 1990, 1992, 1993 were analysed for minimal inhibitory concentrations (MIC) of ampicillin and tetracycline and for plasmid content. MIC values were determined by the agar dilution method and plasmid preparations were performed using alkaline lysis. To detect additional gonococcal strains with *tet(M)* plasmids all strains isolated in 1988–1989 and 1991, in all 234 isolates, were analysed retrospectively for MIC values of tetracycline. If an MIC value of  $\geq 4.0$  mg/l was recorded plasmid analysis was performed.

**Results**—Increased proportions of chromosomally mediated resistance to tetracycline ( $p < 0.001$ ) as well as plasmid mediated resistance to both ampicillin ( $p < 0.02$ ) and tetracycline were found in the later part of the study. In 1991 the first gonococcus with *tet(M)* plasmid was isolated in Sweden. The proportion of strains with chromosomally mediated resistance for ampicillin did not change during the study period. The proportion of gonococcal strains with the 39 kb conjugative plasmid was increased in the later part of the study.

**Conclusions**—The increased proportion of *N gonorrhoeae* strains with resistance to ampicillin and tetracycline is most likely due to importation of strains from areas with high prevalence of antibiotic resistant gonococci. The proportion of *N gonorrhoeae* strains with *tet(M)* plasmids is low in Sweden, but might increase in the same way as the proportion of PPNG strains has increased during 1982–1993.

(Genitourin Med 1995;71:234–238)

**Keywords:** *Neisseria gonorrhoeae*; antibiotic resistance; plasmid

## Introduction

There are two major mechanisms of penicillin and tetracycline antimicrobial resistance in *Neisseria gonorrhoeae*, chromosomally encoded

and plasmid mediated resistance. Chromosomal resistance is encoded by several loci with additive effects<sup>1</sup> and is due to alterations in the penicillin binding proteins and reduced permeability of the outer membrane (for review see <sup>2</sup>). This leads to various degrees of reduced susceptibility to tetracycline and beta-lactam antibiotics. Gonococcal strains with stepwise reduced susceptibility to penicillin were reported already in 1946.<sup>3</sup> Before beta-lactam antibiotics became commonly used in the 1950s the MIC values of the virgin gonococci were  $< 0.1$  mg/l for penicillin.<sup>1</sup> Plasmid mediated resistance to penicillin in gonococci caused by the production of penicillinase (beta-lactamase) was reported in early 1976.<sup>4,5</sup> These penicillinase producing *N gonorrhoeae* (PPNG) strains spread rapidly over the world.<sup>6</sup> The first PPNG strain was reported in Sweden in September 1976.<sup>7</sup> PPNG strains produce a TEM-1 type beta-lactamase which hydrolyses the beta-lactam ring.<sup>8</sup> The PPNG strains mostly carry a beta-lactamase encoding plasmid of African (5.1 kb) or Asian (7.2 kb) type, but other beta-lactamase encoding plasmids have also been reported (for review see <sup>9</sup>). The chromosomally encoded tetracycline resistance is mediated through three different mechanisms; energy dependent efflux of tetracycline, ribosomal protection by reduced binding of tetracycline to the bacterial ribosome, and chemical alteration of the tetracycline molecule (for review see <sup>10</sup>). Resistance to tetracycline was not found when Danish strains from 1944 and 1957 were analysed.<sup>11</sup> Plasmid mediated resistance to tetracycline was first reported in 1985.<sup>12</sup> It was introduced through a conjugative plasmid which carried a *tet(M)* determinant.<sup>13</sup> It encodes for a protein which appears to protect the translation apparatus in an as yet undefined way.<sup>14</sup> *Tet(M)* containing strains have been reported from different parts of the world.<sup>9</sup> Two types of this plasmid have been found; the American (42 kb) and the Dutch (40 kb).<sup>15</sup> It has been suggested that they have different origins.<sup>16</sup> The *tet(M)* plasmid is also able to conjugate beta-lactamase plasmids.<sup>17</sup> Plasmid mediated resistance to ampicillin and tetracycline is generally at a higher level than chromosomally mediated resistance. This study was conducted to investigate the evolution of chromosomal and plasmid mediated resistance to ampicillin and tetracycline in *N gonorrhoeae* strains in Stockholm during 1982–1993.

Department of Microbiology, Stockholm Söder Hospital and Karolinska Institute, Stockholm, Sweden  
M Bäckman

Department of Clinical Microbiology, Karolinska Hospital and Karolinska Institute, Stockholm, Sweden  
K Jacobson  
S Ringertz

Correspondence to:  
M Bäckman, Dep of Microbiology, Stockholm Söder Hospital, S-118 83 Stockholm, Sweden.

Accepted for publication  
1 April 1995

## Materials and methods

### *Patients and gonococcal strains*

Samples from patients attending out-patient clinics both at two general hospitals and from general practitioners rooms in the southern parts of the Stockholm area were included. The catchment area was the same during the study. The proportion of women decreased in the later part of the study and the arithmetic mean of the age increased for both women and men. The proportion of patients with gonorrhoea from STD clinics varied in the beginning of the study, reflecting different strategies of healthcare during that period. In 1982 the proportions of patients from a STD clinic were 78% compared with 35% in 1987. During the later part of the study the proportions of patients from a STD clinic were more stable, in 1990 53%, in 1992 61% and in 1993 56%. The proportions of patients from primary health care centres were 7% in 1982 and 30–40% during the rest of the study. Suspected gonococcal strains were isolated on the basis of colony morphology and positive oxidase test. They were verified by Gram stain and in 1982 fermentation of carbohydrates were included in all verifications. In 1987–1993 co-agglutination (Phadebact, Boule Diagnostics AB, Huddinge, Sweden) was used instead, with the addition of fermentation of carbohydrates if the coagglutination test was negative. All strains included were positive in the coagglutination test. All gonococcal strains in the study were either consecutive or selected randomly from consecutively collected strains by a computer program for randomisation. If more than one strain with the same serovar were isolated from a patient within six weeks only one was included. A total of 404 strains from five different time periods were analysed for MIC values of ampicillin and tetracycline and for plasmid content (table 1). In order to detect *tet*(M) plasmid containing *N. gonorrhoeae* strains all strains isolated from July 1988–February 1990 (174 strains) and from March 1991–February 1992 (60 strains) were analysed retrospectively for MIC values of tetracycline. If more than one strain with the same serovar were isolated from a patient within six weeks only one was included. If the strain had a recorded MIC value of  $\geq 4$  mg/l for tetracycline plasmid analysis was performed.

Table 1 *Neisseria gonorrhoeae* strains analysed for minimal inhibitory concentrations of ampicillin and tetracycline and for plasmid content

Timeperiod of isolation	No of strains selected/ No of strains isolated*	Referred to as
March 1982–Feb 1983	106/905	1982
June 1987–May 1988	104/189	1987
March 1990–Feb 1991	99/99	1990
March 1992–Feb 1993	56/56	1992
March 1993–Feb 1994	39/39	1993

\*If more than one strain with the same serovar were isolated from a patient within six weeks only one was included.

### *Minimum inhibitory concentrations*

The MIC values were determined by the agar dilution method on GC agar base II (BBL, Cockeysville, Maryland, USA) with 1% IsoVitaleX (BBL) and 40 mg/l of haemin (Sigma)<sup>18</sup> containing two-fold dilutions of each antibiotic. Ampicillin sodium (Astra AB, Södertälje, Sweden) and tetracycline hydrochloride (Pharmacia AB, Stockholm, Sweden) were used at concentrations of 0.03–128 mg/l. Ampicillin and not penicillin was chosen since the former was the drug of choice in Sweden. The MIC<sub>50</sub> and MIC<sub>90</sub> values for penicillin and ampicillin do not differ more than one dilution step.<sup>18</sup> All MIC determinations were carried out in duplicate. The mean of the MIC values was calculated for each strain and if it was in between two dilution steps it was adjusted to the nearer higher MIC value. All gonococcal strains had been stored in  $-70^{\circ}\text{C}$ . A total of twenty strains did not grow, these strains did belong to different serovars and did not have any obvious characteristics in common. For MIC determination 100 gonococcal strains were tested together. As controls five gonococcal strains, WHO A, WHO B, WHO C, WHO D and WHO E were used. All isolates having a MIC to ampicillin of  $\geq 2$  mg/l were tested for penicillinase production using chromogenic cephalosporin substrate; Nitrocefin disc test (AB Biodisk, Solna, Sweden). Strains negative in the Nitrocefin test with a MIC value of  $\geq 2.0$  mg/l were defined as having chromosomally mediated resistance to ampicillin (CMRA). Strains positive in the Nitrocefin test were regarded as PPNG. All strains carrying a plasmid of 38–42 kb were analysed for the *tet*(M) plasmid (see below). All isolates without a *tet*(M) carrying plasmid but with a MIC value  $\geq 2.0$  mg/l to tetracycline were regarded as having chromosomally mediated resistance to tetracycline (CMRT).

### *Plasmid DNA preparation, agarose gel electrophoresis and restriction endonuclease digestion*

For the plasmid isolation cells from one haematin agar plate, incubated for 18–24 h, were scraped off with cotton wool swabs and suspended in 2.5 ml 50 mM glucose in TE (25 mM Tris·Cl and 10 mM EDTA, pH 8.0). The cells were harvested and plasmid DNA isolation was performed using alkaline lysis.<sup>19</sup> For the agarose gel electrophoresis 3  $\mu\text{l}$  of plasmid DNA were mixed with 7  $\mu\text{l}$  TE and 2  $\mu\text{l}$  of loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol, 40% sucrose). The electrophoresis (Miniphor Electrophoresis, LKB, Stockholm, Sweden) was performed on 0.8% agarose gel (Seakem, ME, FMC, Invitro, Solna, Sweden) with 0.5  $\mu\text{g}/\text{ml}$  ethidium bromide at approximately 8 V/cm for 2.5 h in TBE (89 mM Tris base, 89 mM boric acid, 2.0 mM EDTA, pH 8.0). The bands were detected on an UV table and photographed. Five gonococcal strains with known plasmids; CCUG 5449, CCUG 6395, CCUG 32095, CCUG 32096 (Culture Collection, University of Gothenburg, Dep of Clin Bact, S-413 46 Gothenburg, Sweden)

Table 2 Distribution of minimal inhibitory concentrations of ampicillin and tetracycline in *N gonorrhoeae* strains isolated at Stockholm Söder Hospital, Sweden

	Minimal inhibitory concentrations of ampicillin*					Minimal inhibitory concentrations of tetracycline*			
	≤ 0.06 No	0.12–0.25 No	0.5–1.0 No	≥ 2.0 CMRA†	> 2.0 beta‡	0.12–0.25 No	0.5–1.0 No	≥ 2.0 CMRT§	≥ 2.0 tet(M)
1982 n = 106	14	33	51	6	2	38	50	18	0
1987 n = 104	22	33	35	8	6	27	54	23	0
1990 n = 99	33	21	31	5	9	11	47	41	0
1992 n = 56	0	23	18	4	11	11	26	18	1
1993 n = 39	10	5	17	2	5	4	12	20	3

\* in mg/l.

† *N gonorrhoeae* strains with chromosomally mediated resistance to ampicillin.

‡ *N gonorrhoeae* strains with beta-lactamase plasmid.

§ *N gonorrhoeae* strains with chromosomally mediated resistance to tetracycline.

|| *N gonorrhoeae* strains with *tet(M)* plasmid.

and *E coli* V512<sup>20</sup> served as controls. Isolates with plasmid bands around 38–42 kb were digested with the restriction endonuclease *Bgl* I (Boehringer Mannheim, Biochemica, Germany) according to the manufacturers instructions. The digested DNA was separated on 0.8% agarose gel with 0.5 µg/ml ethidium bromide.

#### Serological classification

All strains were classified with monoclonal antibodies of the Pharmacia-panel (Boule Diagnostics AB, Huddinge, Sweden). The monoclonal antibodies used for detecting Protein IA were Ar, Ao, As, At, Av and for Protein IB: Br, Bo, Bp, By, Bv, Bu, Bs, Bt, Bx. The WI strains were labelled with an "A" (PrIA) and the WII/III strains with a "B" (PrIB) followed by small letters according to their reactions with the different monoclonal antibody reagents. The antigens were prepared as described.<sup>21</sup> Co-agglutination was performed on bioplates (LabDesign AB, Täby, Sweden).

#### Statistical analysis

Fisher's exact test and the chi square test were used.

#### Results

##### Chromosomally mediated resistance to ampicillin

The distribution of MIC values of non-PPNG strains for ampicillin was similar for the time period 1982–1993 (table 2). In 1982 6% (6/104) of the non-PPNG strains had CMRA compared with 6% (2/34) in 1993. Eighty-four percent (21/25) of all CMRA strains also had CMRT.

##### Plasmid mediated resistance to ampicillin

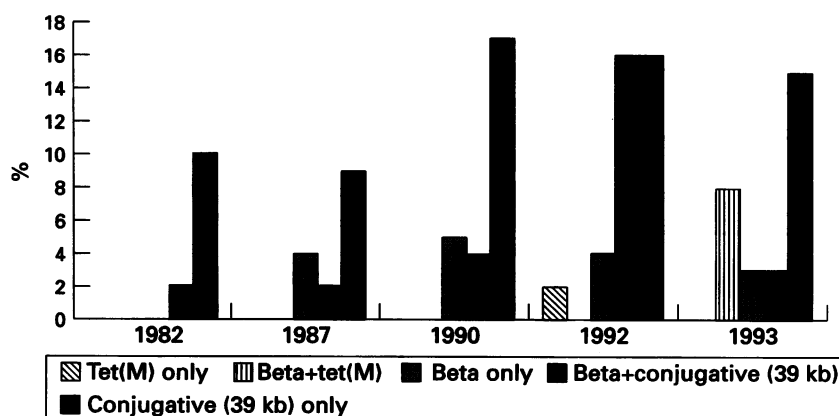
In all, 33 PPNG strains were found in the study. The proportion of PPNG strains rose during the period, with a peak in 1992 (table 2). In 1982, 2% (2/106) of the strains were PPNG and in 1993 13% (5/39) ( $p < 0.02$ ). Beta-lactamase encoding plasmids of both Asian and African types were found. The Asian type dominated in 1982 and 1987, but in 1992 the African type was more frequent. No other types of beta-lactamase encoding plasmids were found. The proportion of PPNG strains with the 39 kb conjugative plasmid was high in 1992 (fig). Seven of these strains harboured the African plasmid and two of the strains harboured the Asian plasmid. The seven strains with African plasmids were of five different serovars. Five of the PPNG strains had a MIC value of 1.0 mg/l for tetracycline, 25 PPNG strains had CMRT and three carried a *tet(M)* plasmid. All strains which produced beta-lactamase according to the Nitrocefin test carried a beta-lactamase encoding plasmid.

##### Chromosomally mediated resistance to tetracycline

The proportions of gonococcal strains with high MIC values to tetracycline increased during the study, especially during the later part (table 2). In 1982 17% (18/106) of the isolates tested had CMRT compared with 56% (20/36) of the non-*tet(M)* strains in 1993, ( $p < 0.001$ ). Eighteen percent (21/120) of all CMRT strains also had CMRA.

##### Plasmid mediated resistance to tetracycline

A total of seven *tet(M)* containing gonococcal strains were found in the study. In April 1991 the first two strains with the *tet(M)* plasmid were isolated from a contact pair. The *tet(M)*



Percentage of *N gonorrhoeae* strains with plasmids, the cryptic plasmid not accounted for.

plasmids were of the American type and the strains were of the serovar Arst. The strains also carried beta-lactamase plasmids of the African type. Another gonococcal strain with the same characteristics was isolated in June 1991. According to the patients the strains originated from Kenya, but there was no known contact between the first contact pair and the patient infected with the third isolate. The fourth *tet(M)* strain of the serovar Arost was isolated in March 1992 and the fifth strain with the serovar Bpyvust in March 1993. These *tet(M)* plasmids were of the Dutch type, acquired in Brazil and Thailand. Another two *tet(M)* strains of Dutch type were isolated in September 1993 on the same occasion from the urethra of a man infected in Nigeria. These strains were of different serovars, Arost and Bpyvu, and carried different types of beta-lactamase encoding plasmids. The only *tet(M)* strain lacking beta-lactamase plasmid had a MIC value of 1.0 mg/l to ampicillin.

#### Other plasmids

The 39 kb conjugative plasmid was found in 60% (18/30) of the non-*tet(M)* PPNG strains compared with 14% (52/370) of the non-*tet(M)* non-PPNG strains ( $p < 0.001$ ). The proportions of non-*tet(M)* non-PPNG strains with the conjugative plasmid was almost doubled between 1987 and 1990 ( $p < 0.06$ , NS) (fig) and remained at the same level in 1992 and 1993. A total of 46/404 strains examined lacked the cryptic plasmid. Forty-one of them were plasmid free and five strains carried the conjugative plasmid only. The cryptic plasmid was more frequent in the later part of the study. Strains of the serovar Brpyust were common among strains without the cryptic plasmid. Sixteen of 35 isolated strains of this serovar lacked the cryptic plasmid ( $p < 0.001$ ). These 16 strains did not harbour any plasmids.

#### Serovars

The 34 strains harbouring plasmids encoding for antibiotic resistance belonged to 18 different serovars. Four serovars were represented by three isolates, eight serovars were represented by two isolates and six serovars by one isolate. Strains belonging to the same serovar carried the same type of beta-lactamase and/or *tet(M)* plasmid, except for the serovar Arst where two types of beta-lactamase plasmids were found in different isolates. It was noted that isolates of the same serovar could differ in the content of the conjugative plasmid.

#### Discussion

This is the first report of *N. gonorrhoeae* strains with *tet(M)* plasmids in Sweden. Increased proportions of *N. gonorrhoeae* strains with beta-lactamase plasmids and chromosomally mediated resistant to tetracycline were also found during 1982–1993. This increase is most likely due to importation from areas with high prevalence of antibiotic resistant gonococci.

In the later part of the present study the proportion of men increased. It has been shown that men more often have imported gonococcal infection than women.<sup>22</sup> The proportion of endemic gonococcal strains in Stockholm decreased by half to 40% during 1987–1989.<sup>23</sup> In another study the majority of patients infected with PPNG strains of known origin were infected abroad, half of them in Asia.<sup>24</sup> High prevalence of PPNG strains has been reported from Southeast Asia.<sup>25–27</sup> Half of all tetracycline resistant *N. gonorrhoeae* strains isolated in Stockholm 1988 were imported from Asia.<sup>22</sup> It has also been reported that a majority of the gonococcal strains isolated in both the Philippines and Thailand in 1989 and 1990 were resistant to tetracycline.<sup>25–26</sup> Tetracycline has never been a drug of choice for treating gonorrhoea in Sweden. However, resistance to tetracycline in *N. gonorrhoeae* strains might also arise in Sweden from treating presumed chlamydial infections and urethritis.

No alterations in the proportions of non-PPNG strains with chromosomally encoded resistance to ampicillin was found during 1982–1993. This stable situation is probably due to a limited pressure from treatment with ampicillin and the distribution has not changed in spite of the increased proportion of imported gonococcal strains. The choice of antibiotic treatment during the study depended on where the patient had contracted the infection. Ampicillin was the drug of choice for treating uncomplicated gonorrhoea in the beginning of this study period but spectinomycin was used if the patient was infected in Asia. During the later part of the study endemic gonorrhoea decreased as the treatment with ampicillin. The quinolones were used together with spectinomycin during the later part of the study since many patients were infected abroad. The evolution into the present level of ampicillin resistance took place some time between 1960 and 1982,<sup>28</sup> most likely in the beginning of the 1960s according to results from Denmark.<sup>11</sup> The distribution of serovars among PPNG and/or *tet(M)* strains in the study implies a minor spread of those strains in Stockholm.

One third of the PPNG strains lacked the 39 kb conjugative plasmid or the *tet(M)* plasmid. These gonococcal strains should not be able to transfer the beta-lactamase producing plasmid and consequently they will not contribute to the spread of this plasmid outside the clone. It has been shown in an *in vitro* experiment<sup>29</sup> that the transfer of the 39 kb conjugative plasmids were not as efficient as the transfer of beta-lactamase encoding plasmids. The proportion of gonococcal strains with 39 kb conjugative plasmid, but without the beta-lactamase encoding plasmid, has increased. Those strains are prepared to conjugate a beta-lactamase encoding plasmid. The frequency of the cryptic plasmid found in this study is in agreement with earlier findings.<sup>30</sup> Gonococcal strains of the serovar Brpyust, lacking the cryptic plasmid, might have represented a clone in the beginning of the 1980s.

The proportions of *N gonorrhoeae* strains with chromosomally mediated resistance to tetracycline increased during the study. The proportion of PPNG strains increased as opposed to that of strains with chromosomally mediated resistance to ampicillin. This might imply that the proportion of gonococcal strains with *tet(M)* plasmids will increase in Sweden in the future, as is the case in other countries.<sup>30</sup> It emphasises the importance of the culturing of *N gonorrhoeae* and the susceptibility testing of all gonococcal isolates. Ampicillin and tetracycline can not be recommended for treating gonorrhoea in Sweden.

Med lab technologist Lena Bengtsson and med lab technologist Margareta Reuter are thanked for skilful technical assistance. We thank ass prof Solgun Bygdeman and ass prof Eric Sandström for critical review of the manuscript. Ass prof Jon Jonasson is thanked for revising the English text. Ampicillin was kindly provided by Astra AB, Södertälje, Sweden and tetracycline by Pharmacia AB, Stockholm, Sweden.

- Cannon JG, Sparling PF. The genetics of the gonococcus. *Ann Rev Microbiol* 1984;38:111-33.
- Spratt BG. Resistance to  $\beta$ -lactam antibiotics mediated by alterations of penicillin-binding proteins. In: Bryan LE, ed. *Microbial Resistance to Drugs*. Springer-Verlag, Berlin, 1989:77-100.
- Franks AG. Successful combined treatment of penicillin-resistant gonorrhea. *Am J Med Sci* 1946;211:553-5.
- Phillips I.  $\beta$ -lactamase-producing, penicillin-resistant gonococcus. *Lancet* 1976;2:656-7.
- Ashford WA, Roman GG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;2:657-8.
- Center for Disease Control. Penicillinase-producing *Neisseria gonorrhoeae*—United States, worldwide. *Morbidity Mortal Weekly Rep* 1979;28:85-7.
- Bygdeman S, Kallings I, Danielsson D. Serogrouping and auxotyping for epidemiological study of  $\beta$ -lactamase-producing *Neisseria gonorrhoeae* strains isolated in Sweden. *Acta Dermatovenereol (Stockh)* 1981;61:329-34.
- Bergström S, Norlander L, Norqvist A, Normark S. Contribution of a TEM-1-like beta-lactamase to penicillin resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1978;13:618-23.
- Roberts MC. Plasmids of *Neisseria gonorrhoeae* and other *Neisseria* species. *Clin Microbiol Rev* 1989;2:18-23.
- Chopra I, Hawkey PM, Hinton M. Tetracyclines, molecular and clinical aspects. *J Antimicrob Chemother* 1992;29:245-77.
- Reyn A, Korner B, Weis Bentzon M. Effects of penicillin, streptomycin, and tetracycline on *N gonorrhoeae* isolated in 1944 and in 1957. *Br J Venereal Dis* 1958;34:227-39.
- Center for Disease Control. Tetracycline-resistant *Neisseria gonorrhoea*—Georgia, Pennsylvania, New Hampshire. *Morbidity Mortal Weekly Rep* 1985;34:563-70.
- Morse SA, Johnson SR, Biddle JW, Roberts MC. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant. *Antimicrob Agents Chemother* 1986;30:664-70.
- Burdett V. Streptococcal tetracycline resistance mediated at the level of protein synthesis. *J Bacteriol* 1986;165:564-9.
- Gascoyne DM, Heritage J, Hawkey PM, Turner A, van Klinger B. Molecular evolution of tetracycline-resistance plasmids carrying *TetM* found in *Neisseria gonorrhoeae* from different countries. *J Antimicrob Chemother* 1991;28:173-83.
- Gascoyne-Binzi DM, Heritage J, Hawkey PM. Nucleotide sequences of the *tet(M)* genes from the American and Dutch type tetracycline resistance plasmids of *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 1993;32:667-76.
- Roberts MC, Knapp JS. Transfer of  $\beta$ -lactamase plasmids from *Neisseria gonorrhoeae* to *Neisseria meningitidis* and commensal *Neisseria* species by the 25.2-megadalton conjugative plasmid. *Antimicrob Agents and Chemother* 1988;32:1430-2.
- Ringertz S, Rylander M, Kronvall G. Disk diffusion method for susceptibility testing of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1991;29:1604-9.
- Extraction and purification of plasmid DNA. In: Sambrook J, Fritsch EF, Maniatis T, eds. *Molecular Cloning: A Laboratory Manual*. Second edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.
- Macrina FL, Kopecko DJ, Jones KR, Ayers DJ, McCowen SM. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. *Plasmid* 1978;1:417-20.
- Bäckman M, Rudén A-KM, Bygdeman SM, Jonsson A, Ringertz O, Sandström EG. Gonococcal serovar distribution in Stockholm, with special reference to multiple infections and infected partners. *Acta Pathol Microbiol Immunol Scand* 1985;Sect B,93:225-32.
- Bygdeman SM, Rudén A-K, Jonsson A, et al. Antibiotic susceptibility, serovars and auxotypes of gonococcal isolates in Stockholm. Relation to geographical origin of the infection. *Int J STD AIDS* 1993;4:33-40.
- Rudén A-K, Jonsson A, Lidbrink P, Allebeck P, Bygdeman SM. Endemic versus non-endemic gonorrhoea in Stockholm: results of contact tracing. *Int J STD AIDS* 1993;4:284-92.
- Rudén A-K. Temporal changes in the gonococcal serovar patterns in Stockholm during two years with special reference to PPNG strains. *Genitourin Med* 1994;70:256-61.
- Clendennen III TE, Hames CS, Kees ES, et al. In vitro antibiotic susceptibilities of *Neisseria gonorrhoeae* isolates in the Philippines. *Antimicrob Agents Chemother* 1992;36:277-82.
- Clendennen TE, Echeverria P, Saengeur S, Kees ES, Boslego JW, Wignall FS. Antibiotic susceptibility survey of *Neisseria gonorrhoeae* in Thailand. *Antimicrob Agents Chemother* 1992;36:1682-7.
- Kam K-M, Lai C-F, Egglestone S, Chan C-BB. Patterns of antibiotic susceptibility of gonococci isolated in Hong Kong, 1987-1990. *Sex Transm Dis* 1992;19:284-7.
- Ringertz O. The sensitivity of *N gonorrhoeae* to antibiotics. *Acta Pathol Microbiol Scand* 1961;53:173-9.
- Roberts M, Falkow S. Plasmid-mediated chromosomal gene transfer in *Neisseria gonorrhoeae*. *J Bacteriol* 1978;134:66-70.
- Roberts M, Piot P, Falkow S. The ecology of gonococcal plasmids. *J Gen Microbiol* 1979;114:491-4.